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青蒿素在露水草毛状根中的生物转化*

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摘要　露水草（*Cyanotis arachnoidea* C. B. Clarke）毛状根培养系中加入青蒿素培养 8 d 后，青蒿素转化为去氧青蒿素。根据光谱数据，对去氧青蒿素的结构进行了鉴定。结果表明，通过露水草毛状根能将青蒿素进行选择性还原为去氧青蒿素。

关键词　青蒿素，去氧青蒿素，露水草，生物转化，毛状根
分类号　Q946

Biotransformation of Artemisinin by Hairy Roots of Cyanotis arachnoidea

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Abstract　The biotransformation of artemisinin by the hairy roots of *Cyanotis arachnoidea* was investigated. After incubation for 8 days, the main product was determined as deoxyartemisinin.

Key words Artemisinin, Deoxyartemisinin, *Cyanotis arachnoidea*, Biotransformation, Hairy roots

The ability of plant hairy roots to convert foreign substances into potentially useful substances is of great interest, as products may be formed which are difficult to be prepared by synthetic methods (Pras, 1992). Artemisinin (**1**), a sesquiterpene lactone with a 4,6-endoperoxide function, is a very potent antimalarial principle isolated from the Chinese medicinal herb *Artemisia annua* L. (Compositae) (Liu *et al*, 1979). Now, It is used in the treatment of malaria as a potent therapeutic agent. It has been shown that this natural product has remarkable anti-HIV and anti-schistosome activities. Thus biosynthesis of artemisinin is obvious important. This paper deals with the biotransformation of **1** by hairy roots of *Cyanotis arachnoidea* C. B. Clarke (Commelinaceae).

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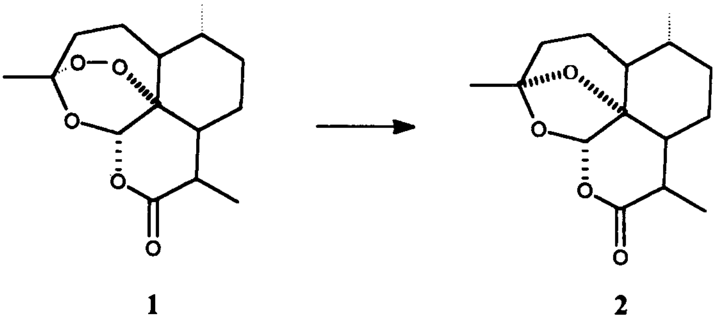

Fig. 1 Reduction of **1** to **2** by hairy roots of *C. arachnoidea*

RESULTS AND DISCUSSION

Incubation of **1** with the hairy roots of *C. arachnoidea* gave deoxyartemisinin **2** as the main biotransformation product (Fig. 2c). No formation of biotransformation products were observed in the control experiment without substrate (Fig. 2d). In time course experiments (Fig. 3), the formation of **2** reached its maximum after 8 days of incubation and then decreased gradually.

In order to isolate the compounds from cultured system, a large scale incubation of **1** with the hairy roots of *C. arachnoidea* was carried out. After the biotransformation, both **1** and **2** were extracted and purified as described in the Experimental. The structures of the compounds **1** and **2** were determined by spectral methods. Both **1** and **2** were indentified as artemisinin and deoxyartemisinin by comparison with literature data (Liu *et al*, 1979; Tu *et al*, 1981). It was considered that **1** was transformed to **2** by high selective reduction.

In 1980, Zhu *et al* (1980) reported the reduction of **1** to **2** in human body. To our knowledge, the root system of *C. arachnoidea* is only one example of monocotyledous plants for inducing hairy roots up to now (Zhou *et al*, 1996). This is the first report of the reduction of **1** to **2** with plant hairy roots. We also tested *Panax japonicus* var. *major* hairy roots and *Nicotiana tabacum* callus cultures with **1**, but no conversion was observed. This suggests that the hairy roots of *C. arachnoidea* could be used for selective reduction of peroxides.

EXPERIMENTAL

General Mps: uncorr; IR: KBr; MS were obtained by direct inlet at 70 eV, ^{1}H and ^{13}C NMR were recorded at 400 MHz NMR spectrometer, and chemical shifts are given as δ (ppm) with tetramethylsilane (TMS) as an internal standard. For HPLC analysis of **1** and **2**, the mobile phase was MeOH–H_2O (8:2). The flow rate was 0.5 mL/min. A Shimadzu LC–4A HPLC system was used, and it consisted further of a reversed phase column (Partisil ODS 2, 10μ C–18, 250 mm× 4.6 mm, Alltech, France), a SPD–2AS UV–detector, a Shimadzu C–R3A data processor. The compounds were detected at wavelength 210 nm.

Plant material A hairy root system of *C. arachnoidea* C. B. Clarke has been established from its intact plants by using a wild strain pRi 15834 of *Agrobacterium rhizogenes* (Zhou *et al*, 1996). Hairy roots free from *A. rhizogenes* were established in MS (Murashige and Skoog, 1962) liquid medium without

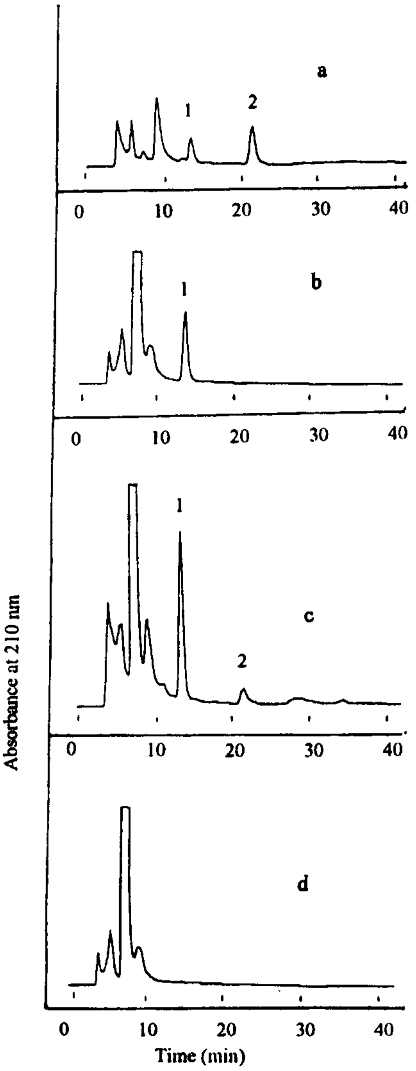

Fig. 2 HPLC of artemisinin (1) and deoxyartemisinin (2). (a) Standard of 1 and 2; (b) Beginning of feeding experimental; (c) 8 days period of incubation after 1 was added into media; (d) 8 days period of incubation after only water was added into media. The chromatographic conditions are described in the Experimental. Peak 1 (Rt = 13.47 min) is the administered artemisinin. Peak 2 (Rt = 21.39 min) is the transformation product deoxyartemisinin.

phytohormone. They were cultivated in 250 mL Erlenmeyer flasks containing 100 mL medium on a gyratory shaker (110 r/min) in dark at 22±1℃. Every 14th day a leading root tip was transferred into fresh medium.

Time course experiments: 660 mg of artemisinin (20 mg/100 mL of medium in a 250 mL volume Erlenmeyer flask) was added into the medium after the hairy roots of *C. arachnoidea* were cultured for 2 weeks. Time course experiments were conducted over an incubation period of 12 days at pH 5.75. After the incubation, the medium was extracted with $CHCl_3$, and the hairy roots were freeze—dried and extracted with $CHCl_3$. The $CHCl_3$ extract was dissolved in MeOH, filted and made up to 2 mL. About 10 μL of the soln was injected to HPLC analysis. The amounts of **1** and **2** were determined on the basis of the peak area from HPLC. The correlation equation for **1** was $y = -1308.4 + 1056.8x$, here y was peak area, x was quantity of **1** for every injection, and correlation efficient was 0.998. The correlation equation for **2** was $y = -2377.1 + 2363.4x$, here y was peak area, x was quantity of **2** for each injection, and correlation efficient was 0.999.

Isolation and indentification of products 300 mg of **1** (20 mg/100 mL of medium) was added into the medium after the hairy roots were cultured for 2 weeks. At the end of the incubation period of 8 days, the culture medium was collected and extracted with $CHCl_3$. The hairy roots were also collected, freeze—dried, and extracted with $CHCl_3$, the $CHCl_3$extracts were combined and the solvent removed under reduced pressure to give a residue (700 mg). The extracts were separated by column chromatography and preparative thin layer chromatography over silica gel, to provide **1**(104 mg) and **2** (62 mg).

Artemisinin (**1**): $C_{15}H_{22}O_5$, needles (in $CHCl_3$), mp. 156～157℃; $IR\nu_{max}^{KBr}cm^{-1}$: 1745, 1115, 881, 831; EIMS m/z: 282[M]$^+$, 264, 250, 236, 222, 207, 192, 178, 166, 151, 137, 123, 107; ^1H NMR (400 MHz, $CDCl_3$): δ 5.83 (1H, s, H—5), 3.36 (1H, dq, J = 5.7, 7.2 Hz, H—11), 2.4 (1H, dt, J = 5.84, 14.64 Hz, H—7), 1.40 (3H, s, H—15), 1.17 (3H, d, J = 7.28 Hz, H—13), 0.97 (3H, d, J = 5.92 Hz, H—14); ^{13}C NMR (400 MHz, $CDCl_3$): δ 50.2 (C—1), 24.9 (C—2), 36.0 (C—3), 105.4 (C—4), 93.7

(C—5), 79.5 (C—6), 45.1 (C—7), 23.4 (C—8), 33.7 (C—9), 37.6 (C—10), 32.9 (C—11), 171.9 (C—12), 12.5 (C—13), 19.8 (C—14), 25.2 (C—15). (Liu *et al*, 1979; Tu *et al*, 1981; Huang *et al*, 1987).

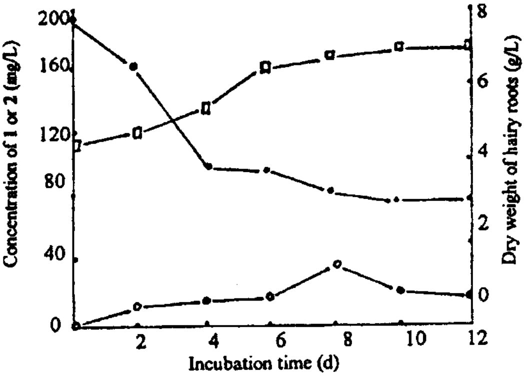

Fig. 3 Time course of the biotransformation of **1** to **2** by hairy roots of *C. arachnoidea*.

(●): 1; (○): 2; (□): dry weight of the hairy roots.

Deoxyartemisinin (**2**) : $C_{15}H_{22}O_4$, needles (in $CHCl_3$), mp 109～110℃ ; IRν_{max}^{KBr}cm^{-1}: 1750; EIMS m/z: 266[M]$^+$, 238, 222, 210, 195, 179, 165, 151, 140, 135, 124, 107; ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.67 (1H,s, H—5), 3.16 (1H, dq, J = 4.6, 7.2 Hz, H—11), 1.98 (1H, dt, J = 4.36, 13.0 Hz, H—7), 1.50 (3H, s, H—15), 1.17 (3H, d, J = 7.28 Hz, H—13), 0.91 (3H, d, J = 5.92 Hz, H—14); ^{13}C NMR (400 MHz, $CDCl_3$): δ 44.7 (C—1), 23.6 (C—2), 34.0 (C—3), 109.2 (C—4), 99.7 (C—5), 82.5 (C—6), 42.5 (C—7), 22.1 (C—8), 33.5 (C—9), 35.4 (C—10), 32.8 (C—11), 171.7 (C—12), 12.6 (C—13), 18.5 (C—14), 24.0 (C—15).(Liu *et al*, 1979; Zhu *et al*, 1980; Huang *et al*, 1987).

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